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Hemodynamic and renal effects of bosentan and losartan in 2k1c hypertensive rats

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Abstract

Endothelin-1 (ET-1) and Angiotensin II (Ang II) play important roles in generating hypertension. The present work was investigated the hemodynamic and renal effects of both ET-1 and Ang II on 2K1C rats. The design of this study included five groups of rats: Group 1 Sham rats, Group 2 Normotensive rats ($2K1C_{(n)}$), Group 3 two kidney one clip hypertensive rats ($2K1C_{(h)}$), Group 4 two kidney one clip hypertensive rats + Bosentan ($2K1C_{(h)}$ + Bosentan), and Group 5 two kidney one clip hypertensive rats + Losartan ($2K1C_{(h)}$ + Losartan). Systolic blood pressure (SBP) was significantly elevated in $2K1C_{(h)}$ rats from third to sixth week after surgery compared with control rats. SBP significantly fell in bosentan and losartan treated rats. Serum nitric oxide (NO) level in hypertensive rats was slightly decreased, whereas in losartan group it was significantly increased. Serum malonaldehyde (MDA) significantly increased in bosentan treated rats as compared to hypertensive rats. Bosentan administration caused a markedly increased in Na^+ excretion rate compared with $2K1C_{(h)}$ rats in week four after surgery. In conclusion, losartan is more potent than bosentan in reducing hypertension via elevation of serum NO level.

Introduction

Hypertension (HTN) has an important contribution in cardiovascular risks factors. It has been considered by the researchers. Several animal models have been used to investigate the consequences of HTN. The most well-known model is Goldblatt model, which is widely used for renovascular HTN (1).The 2K1C Goldblatt model is induced by using silver clip on unilateral stenosis of the renal artery and the other kidney is untouched (2).

The definitive place of ET-1 in the hypertension has been a controversial subject among researchers over the last two decades since the discovery of ET-1 (3, 4). Schiffrin (5) reported that plasma ET-1 concentration is elevated in essential hypertension, and it leads to constrict vascular beds, increases smooth muscle hypertrophy, proliferation and rises vascular resistance.

ET has many receptor subtypes. They are participated greatly in physiology process; so scientists are attempted to block ET-1 signal receptors by peptide and non-peptide substances to understand their actions on the body. Davenport (6) revealed that ET-1 receptor antagonists are currently classified as ET_A selective (BQ 123), ET_B selective (BQ 788), and mixed antagonists that display similar affinities for both receptor subtypes. Both of ET_A selective and ET_A/ ET_B (Bosentan) antagonists are currently evaluated in clinical trials.

Beside ET-1, Ang II has great roles in cardiovascular diseases. It also has two subtype receptors AT_1 via G protein coupling receptor constricts blood vessels and AT_2 via production cGMP produce NO (7). Ang II

receptors are widely distributed in the body organ tissues, but they are abundant in the nephrons. AT_1 activation decreases pressure natriuresis and diuresis. A constriction character of Ang II on blood vessels as like as ET-1 leads to produce hypertension (8). Little is known about the physiological actions of ET-1, Ang II and their antagonists on 2K1C induced hypertensive rats. So the present study aimed to investigate the hemodynamic and renal effects of ET-1 and Ang II on 2K1C rats.

Materials and Methods

Animals

Thirty nine male rats were used their body weights between 200 - 400 grams. This experiment was conducted on male due to female resistance to hypertension (9). The employed experimental animals were met the criteria of ethic rules of the supervising committee of Biology department, College of Science, Salahaddin University. Rats were given standard rat diet, when room temperature was controlled in range 22 ± 2 ◦C and 12 / 12 dark cycle photoperiod.

Experimental design

This experiment was designed to develop renovascular hypertensive rats through two kidney one clip (2K1C) Goldblatt model, then treating them by bosentan and Losartan. To obtain the goal of experiment five groups were performed under same house conditions. Group 1 (Sham rats, n = 9), rats underwent left abdominal incision and all the surgical procedure without using silver clip, they had been given one ml normal saline (0.9%) by gavage every day; Group 2, $2K1C_{(n)}$ rats (n = 7) served as a negative control in which rats had a silver clip around the left renal artery, they would not develop hypertension; Group 3, $2K1C_{(h)}$ rats (n = 7) served as a positive control in which rats had a silver clip around left renal artery with developed hypertension; Group 4, $2K1C_{(h)}$ + Bosentan rats (n = 8) rats had silver clip around left renal artery with hypertension, then treated by bosentan 30 mg / kg (Cipla, India) every day by gavage, after two weeks of surgery from developed hypertension continued for four weeks (10); Group 5, $2K1C_{(h)}$ + Losartan (n = 8) involved rats had silver clip around left renal artery developed hypertension then Losartan (Actavis, Icelanda) was administrated by gavaging 30 mg / kg every day, after two weeks of surgery from developed hypertension continued until four weeks.

Preparation of animals for 2K1C Goldblatt hypertension

Animals were anesthetized by injection with a mixture of Ketamine hydrochloride 80 mg / Kg (Trittau, Germany) and Xylazin 12 mg / Kg (Interchem, Holland) intraperitonealy(11). Left abdominal side was cleaned from fur; a 4 – 5 cm flank incision was made to expose the left kidney, then it was carefully dissected and freed left renal artery from left renal vein. An internal diameter (0.25 - 0.3) mm silver clip was placed around the left renal artery which it was caused partial occlusion, and a 70% of blood flow was decreased. After performing clipping process successfully, the left kidney was slowly pushed back into the retroperitoneal cavity, the wound was cleaned by sterilized solution, the abdominal muscle was sutured by 3.0 gage chromic gut suture and the abdominal skin sutured by ethilon monofilament nylon suture 3.0 gage. The whole procedures on sham group had been done without clipped silver clip around left renal artery.

Determination of noninvasive systolic blood pressure

Conscious rats were pre warmed till 30 - 40 ◦C in paper box. SBP was measured by tail cuff method (12). The electro sphygmomanometer (AD Instruments, Power Lab 2 / 25, software LabChart6) was used. Rat's tail was inserted into cuff and transducer pressed on caudal artery during inflation; SBP was represented as the average of 5 consistent readings.

Determination of serum NO

Griess reagent system (sulfanilamide and N-1-napthylethylenediamine dihydrochloride) was used to determine serum NO under acidic (phosphoric acid) conditions. 0.5 ml serum sample was added into clean centrifuge test tube (Supe-Rior,W-Germany), to precipitate serum protein by adding 300 µL of 0.15 M $ZnSO_4$ (13), neutralized media by adding 10 µL of 10 M NaOH (14), Mixed well by vortex (Vortex-Genie, Model K - 550-Ge, USA). It was cooled on ice for 15 minutes and centrifuged (Centromix-Mod. S - 549) at 1000 g for 10 minutes. Supernatant 0.5 ml was transferred into clean labeled plastic test tube (Screw tube), to reduce nitrate (NO_3) to nitrite (NO_2), 2 - 3 copper cadmium granules were added. After that, 0.5 ml supernatant was added into another clean labeled plastic test tube and 0.5 Griess reagent acid was added into each sample, then mixed and incubate at room temperature for 15 min. The violet colour reagent was read at 543 nm.

Determination of serum MDA

Malondialdehyde is a byproduct of lipid peroxidation. It was reacted with Thiobarbituric acid (TBA) to produce a complex MDA - TBA. This complex was determined spectrophotometrically at 520 nm.

Determination of serum glucose

Oxidation glucose by many enzymes, which performed in a Kit (Randox) produced a violet quinoneimine coluor absorbed by spectrophotometer (500 nm) which was proportioned to glucose concentration

AST and ALP enzyme activities determinations

AST and ALP are considered as liver function test parameters; they are measured by applying automated biochemical analyzer (Cobas,USA)

Determination of sodium excretion rates

Excretion rates were found by calculation from urine flow and electrolyte concentration in the urine as the following equation

Sodium excretion rate (mEq / hr / Kg) = Urine flow (ml / hr / Kg b.w) * Na^+ concentration in urine mEq / L

Statistical analysis

All data were expressed as means ± standard error (SE) and statistical analysis was carried out by using statistical package for social science social version 16 (SPSS). One way analysis of variance and post hoc Duncan were used to analysis data. P values < 0.05 were considered as significant.

Results

Fig 1 shows that SBP were significantly ($P < 0.001$) elevated in $2K1C_{(h)}$ rats from third to sixth week respectively after surgery as compared with sham operated rats. There was also a significant decrease in normotensive rats compared with hypertensive rats. There was not significant difference in week three but by week four SBP had significantly fallen in bosentan treated rats compared with hypertensive rats. Also, administration of losartan was significantly lowered SBP.

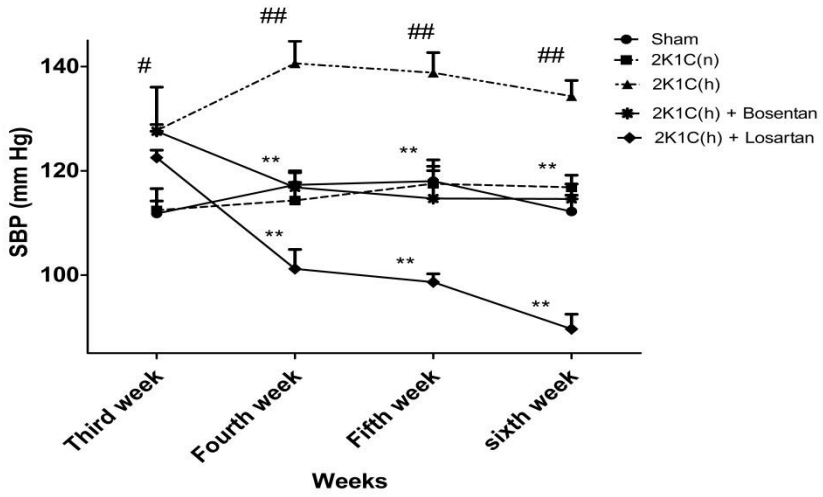

Figure 1: Effects of bosentan and losartan on systolic blood pressure (mm Hg) in $2K1C_{(h)}$. $2K1C_{(n)}$, two kidney one clip normotensive rats; $2K1C_{(h)}$, two kidney one clip hypertensive rats; $2K1C_{(h)}$ + Bosentan, two kidney one clip hypertensive rats plus bosentan administration; $2K1C_{(h)}$ + Losartan, two kidney one clip hypertensive rats plus losartan administration. Data are expressed mean ± standard error. $^{\#}P < 0.05$ vs $2K1C_{(n)}$ and $^{*}P < 0.05$ vs $2K1C_{(h)}$ one way ANOVA (CRD) and post - hoc Duncan

As shown in Table 1, serum NO level in hypertensive rats was slightly decreased (6.457 ± 0.8242) compared to sham rats (8.900 ± 1.291). Serum NO level of losartan group was significantly ($P < 0.05$) increased (15.37 ± 3.704) as compared with hypertensive rats. Besides, serum NO level in bosentan groups was raised (8.125 ± 1.544) but not significantly consideration. There was no significant difference between normotensive rats and hypertensive rats. Serum MDA concentration significantly ($P < 0.05$) increased in bosentan treated rats (6.881 ± 0.7599), as compared with hypertensive ones. Also, in losartan treated rats serum MDA level (4.848 ± 0.4022) increased, but not significantly in comparison with hypertensive rats (3.766 ± 0.2789). Serum MDA in normotensive and hypertensive groups did not change statistical significant as compared with sham group (Table).

In addition, AST and ALP tended to increase in both $2K1C_{(h)}$ and $2K1C_{(n)}$ groups (Table). Losartan administration was not significantly lowered serum AST while, ALP activity significantly decreased (144.7 ± 7.946, and 187.8 ± 10.57), respectively as compared with $2K1C_{(h)}$ rats (160.5 ± 5.830, and 225.4 ± 16.29) (Table). Thus, bosentan lowered ALP activity, whereas, it further increased serum AST activity in $2K1C_{(h)}$ treated rats (Table). Besides that, glucose concentration in serum of normotensive rats significantly increased (167.3 ± 12.62) ($P < 0.05$) in comparison with hypertensive rats (127.1 ± 11.11). The hypertensive rats did not differ significantly from sham rats. There are no statistically significant differences appeared between losartan and bosentan effects in hypertensive rats (Table). As shown in (Fig 2), bosentan administration caused a marked ($P < 0.05$) increase in sodium excretion rate compared with $2K1C_{(h)}$ rats in week four after surgery. Losartan administration slightly increased sodium excretion rate compared to $2K1C_{(h)}$ group.

Table: Effects of bosentan and losartan on serum NO (µmol / L), MDA AST, ALP activity, and Glucose from third week to sixth week after surgery in 2K1C hypertensive rats

Parameters **Groups**	**NO *** **(µmol / L)**	**MDA *** **(µmol / L)**	**AST *** **(U / L)**	**ALP *** **(U / L)**	**Glucose *** **(mg / dL)**
Sham	8.900 ± 1.291^{a}	4.038 ± 0.1459^{ab}	135.6 ± 4.350^{a}	114.9 ± 9.322^{a}	119.5 ± 4.455^{a}
2K1C$_{(n)}$	6.600 ± 0.8242^{a}	3.052 ± 0.1725^{a}	184.0 ± 8.113^{bc}	214.1 ± 13.23^{bc}	167.3 ± 12.62^{b}
2K1C$_{(h)}$	6.457 ± 1.038^{a}	3.766 ± 0.2789^{ab}	160.5 ± 5.830^{ab}	225.4 ± 16.29^{c}	127.4 ± 11.11^{a}
2K1C$_{(h)}$ + Bosentan	8.125 ± 1.544^{a}	6.881 ± 0.7599^{c}	213.3 ± 29.96^{c}	179.2 ± 12.36^{b}	129.3 ± 9.508^{a}
2K1C$_{(h)}$ + Losartan	15.37 ± 3.704^{b}	4.848 ± 0.4022^{b}	144.7 ± 7.946^{a}	187.8 ± 10.57^{b}	143.7 ± 16.86^{ab}

Two kidney one clip normotensive rats were compared with two kidney one clip hypertensive rats. 2K1C$_{(n)}$, two kidney one clip normotensive rats; 2K1C$_{(h)}$, two kidney one clip hypertensive rats; 2K1C$_{(h)}$ + Bosentan, two kidney one clip hypertensive rats plus bosentan administration; 2K1C$_{(h)}$ + Losartan, two kidney one clip hypertensive rats plus losartan administration. The different letters mean significant and the same letters mean no significant differences. The data mean ± SEM * $P < 0.05$ considered a significant difference according to 1 - way ANOVA followed by Duncan post hoc test

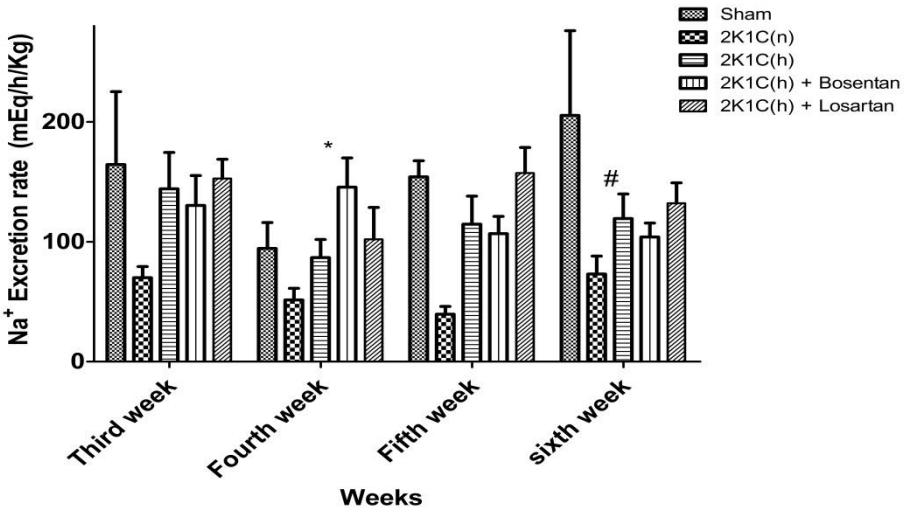

Figure 2: Effects of bosentan and losartan on sodium excretion rate (mEq / h / Kg) in 2K1C$_{(h)}$. 2K1C$_{(n)}$, two kidney one clip normotensive rats; 2K1C$_{(h)}$, two kidney one clip hypertensive rats; 2K1C$_{(h)}$ + Bosentan, two kidney one clip hypertensive rats plus bosentan administration; 2K1C$_{(h)}$ + Losartan, two kidney one clip hypertensive rats plus losartan administration. Data are expressed mean ± standard error. $^{\#}P < 0.05$ vs 2K1C$_{(n)}$ and $^{*}P < 0.05$ vs 2K1C$_{(h)}$ one way ANOVA (CRD) and post - hoc Duncan

Discussions

Experimentally had been showed that 2K1C model did not elevate BP in the first and second weeks after surgery, but from week three after surgery, BP elevated significantly ($P < 0.05$), and it was remained on elevation until week six post-surgery (Fig 1). It means that 2K1C model for development of hypertension is highly associated to the time (15). Also, it was related to salt and water retention, which they were retuned slowly at the beginning (1) It was similar to our data regarding urine Na^+ excretion rate which were decreased in hypertensive rats (Fig 2).

On the other hand, Renin angiotensin aldosterone system and Frank-Starling mechanism were highly contributed in rising BP in 2K1C model by increasing blood volume and rose volume load (8). Also, rising of free radicals was participated in hypertension (16). Administration of bosentan could decrease BP significantly ($P < 0.05$) in 2K1C hypertensive rats. The mechanism of decreasing blood pressure by bosentan is due to blocking of $ET_{A/B}$ receptors, which leads to decrease in peripheral resistance (6). Also, the results showed that bosentan increased Na^+ excretion rate, through bosentan contributions in decreasing salt and water retention. Losartan administration significantly ($P < 0.05$) decreased the elevated BP (Fig 1), because losartan blocks AT_1 receptor that prevents Ang II constriction character on blood vessels, but AT_2 receptor (17) continuously elevates serum NO concentration (Table) which is a potent vasodilator substance that decreases peripheral resistances (17).

Furthermore, many rats of the present study were followed up from day of surgery till six weeks and BP was measured once a week their BP did not exceed more than 125 mm Hg. They were considered as normotensive (Fig 1). The mystery behind 2K1C became normotensive related to the strain of rat (18). Also, it was related to slow responsiveness to presser effect of Ang II; the arterial pressure response to renal ischemia may depend on both AngII formation and responsiveness to the chronic actions of the peptide (19).

The data was showed that, NO changed among groups but not reached the level of significance except in losartan group (Table). The well established mechanism for this circumstance, 2K1C model is renin angiotensin system dependent. Ang II is directly increased free radicals, which they are reacted with NO, and then it is decreased serum NO concentration. When, losartan administrated orally it was caused an increases in serum NO due to blocks AT_1 receptor (20). To understand the role of free radicals in hypertension, MDA has been measured as indicator of the level of free radicals. The result showed that, bosentan had harmful risk effects of the body due to rose MDA level (Table), it also has hepatic toxicity and liver damage (21), but the exact mechanism by which bosentan increases free radicals is not fully understood, so further research needs to confirm this finding and explain the possible physiological role of bosentan risk factors. AST activity was in the serum measured; it was highly activity in the serum of bosentan group (Table). It means that bosentan affected liver cells, and damaged them, as well as increased MDA (22)

$2K1C_{(h)}$ model could increase LFT markedly, especially in ALP activities (Table). The mechanisms for increasing LFT may be related to the elevation of serum Ang II. It has been directly caused liver fibrosis, hepatic cell proliferation, and inflammatory cytokine release (23). Also, Ang II is indirectly increased liver enzymes production through increasing portal vein (24). So hypertension indirectly causes an increase in liver enzymes (25)**.** Bosentan administration, despite its hypotensive effects, increased AST activity significantly ($P < 0.05$) (Table), because bosentan itself is reported to cause hepatic toxicity and liver damage (21). In contrast to bosentan, losartan administration decreased AST and ALP significantly ($P < 0.05$). These interested findings are confirmed by Sookoian (26). They experimentally demonstrated that losartan has antifibrosis action on liver cells and other organs. Our results showed that AST and ALP in normotensive rats increased in comparison with sham rats. Until now, there is not clear mechanism to interpret that, but our study observed of clipped kidney; substances from fibrosis may enhance liver cells to increase liver enzyme activities.

The 70% occlusion of blood flow from left renal artery causes decreased Na^+ excretion rate of the hypertensive rats. They are increased in bosentan and losartan treated rats compared with $2K1C_{(h)}$ rats (Fig 2). Although our results did not reach significant level in all time intervals, but still it was parallel with other studies which

reported that, clipping method would cause decreasing pressure natriuresis and diuresis, because Ang II stimulates adrenal cortex to increase aldosterone secretion. It is enhanced Na^+ and water retention by activating Na^+ - K^+ ATPase pump and increasing Na^+-water co-transport (1). The most harmful risk of cardiovascular disease is hyperglycemia, so that for the first time our results observed that $2K1C_{(n)}$ rats had markedly elevated serum glucose as compared with sham. According to our knowledge, there is no report correlating such resistance of hypertension in $2K1C_{(h)}$ rats with high blood glucose. Whether unchanged right kidney weight when left kidney have been clipped has a role or not, it has not been explained yet. Such unexplainable results need further confirmation.

Conclusions

Two kidney one clip for developing hypertension is required sufficient time at least two weeks. Losartan was more potent than bosentan in reducing hypertension through elevation of serum NO level. Bosentan was increased Na^+ excretion rate. Many rats had resistance for 2K1C Goldblatt model, but with the resistance character they were became hyperglycemia.

References

1. Baydal, D.K., Lata, H. and Dadhich, A.P. "*ANIMAL MODELS OF HYPERTENSION AND EFFECT OF DRUGS"*, Indian Journal of Pharmacology, Vol. (35), pp. 349-62. (2003).

2. Chabielska, E., Matys, T., Kucharewicz, I., Pawlak, D., Rolkowski, R. and Buczko, W. "*The involvement of AT(2)-receptor in the antithrombotic effect of losartan in renal hypertensive rats"*, J Renin Angiotensin Aldosterone Syst, Vol. (1), No. (3), pp. 263-7. (2000).

3. Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., and Mitsui, Y. "*A novel potent vasoconstrictor peptide produced by vascular endothelial cells"*, Nature, Vol. (332), No. (6163), pp. 411-5. (1988).

4. Dhaun, N., Goddard, J., Kohan, D.E., Pollock, D.M., Schiffrin, E.L. and Webb, D.J. "*Role of endothelin-1 in clinical hypertension: 20 years on"*, Hypertension, Vol. (52) No. (3), pp. 452-9. (2008).

5. Schiffrin, E.L., Lariviere, R., Li, J.S., Sventek, P. and Touyz, R.M. "*Deoxycorticosterone acetate plus salt induces overexpression of vascular endothelin-1 and severe vascular hypertrophy in spontaneously hypertensive rats"*, Hypertension, Vol. (25), No. (4 Pt 2), pp. 769-73. (1995).

6. Davenport, A.P. and Battistini, B. "*Classification of endothelin receptors and antagonists in clinical development"*, Clin Sci, Vol. (103), No. (48), pp. 1S-3S. (2002).

7. Gilliam-Davis, S., Gallagher, P.E., Payne, V.S., Kasper, S.O., Tommasi, E.N. and Westwood, B.M. "*Long-term systemic angiotensin II type 1 receptor blockade regulates mRNA expression of dorsomedial medulla renin-angiotensin system components"*, Physiol Genomics, Vol. (43) No. (13), pp. 829-35. (2011).

8. Barrett, K.E., Barman, S.M. and Boitano, S. *"Ganong's review of medical physiology"*, Brooks HLTteM-H, United States of America. (2010).

9. Cotter, J.L., Vandongen, R. and Burton. D.L. "Sturm MJ. Platelet activating factor and one-kidney, one clip hypertension", Hypertension, Vol. (15) No. (6 Pt 1), pp 628-32. (1990).

10. Lee, T.M., Lin, M.S. and Chang, N.C. "*Physiological concentration of 17beta-estradiol on sympathetic reinnervation in ovariectomized infarcted rats"*, Endocrinology, Vol. (149) No. (3), pp. 1205-13. (2008).

11. Thone-Reineke, C., Olivier, J., Godes, M., Zart, R., George, I. and Bauer, C. "*Effects of angiotensin-converting enzyme inhibition and calcium channel blockade on cardiac apoptosis in rats with 2K1C (two-kidney/one-clip) renovascular hypertension"*, Clin Sci, Vol. (104) No. (1), pp. 79-85. (2003).

12. De Nicola, L., Blantz, R.C. and Gabbai, F.B. "*Nitric oxide and angiotensin II. Glomerular and tubular interaction in the rat"*. J Clin Invest, Vol. (89), No. (4), pp. 1248-56. (1992).

13. Huskic, J., Culo, F., Dautovic, S. and Mulabegovic, N." *Angiotensin converting enzyme activity and nitric oxide level in serum patients with dehydration",* Bosn J Basic Med Sci, Vol. (7), No. (1), pp. 33-6. (2007).
14. Xiong, Y., Fu, Y.F., Fu, S.H. and Zhou, H.H. "*Elevated levels of the serum endogenous inhibitor of nitric oxide synthase and metabolic control in rats with streptozotocin-induced diabetes",* J Cardiovasc Pharmacol, Vol. (42) No. (2), pp. 191-6. (2003).
15. Helle, F., Vagnes, O.B, and Iversen, B.M. "*Angiotensin II-induced calcium signaling in the afferent arteriole from rats with two-kidney, one-clip hypertension"* Am J Physiol Renal Physiol, Vol. (291), No. (1), pp.7. (2006).
16. Bedard, K. and Krause, K.H. "*The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology",* Physiol Rev, Vol. (87), No. (1), pp. 245-313. (2007).
17. Yan, C., Kim, D., Aizawa, T. and Berk, B.C. "*Functional interplay between angiotensin II and nitric oxide: cyclic GMP as a key mediator"* Arterioscler Thromb Vasc Biol, Vol. (23), No. (1), pp. 26-36. (2003).
18. Pinto, Y.M., Paul, M. and Ganten, D. "*Lessons from rat models of hypertension: from Goldblatt to genetic engineering*", Cardiovasc Res, Vol. (39), No. (1), pp. 77-88. (1998).
19. Melaragno, M.G. and Fink, G.D. "*Slow pressor effect of angiotensin II in normotensive rats with renal artery stenosis",* Clin Exp Pharmacol Physiol, Vol. (23), No. (2), pp.140-4. (1996).
20. Benz, J., Oshrain, C., Henry, D., Avery, C., Chiang, Y.T. and Gatlin, M. "*Valsartan, a new angiotensin II receptor antagonist: a double-blind study comparing the incidence of cough with lisinopril and hydrochlorothiazide",* J Clin Pharmacol, Vol. (37), No. (2), pp. 101-7. (1997).
21. Eriksson, C., Gustavsson, A., Kronvall, T. and Tysk, C. "*Hepatotoxicity by bosentan in a patient with portopulmonary hypertension: a case-report and review of the literature"* J Gastrointestin Liver Dis, Vol. (20), No. (1), pp.77-80. (2011).
22. Rich, S. and McLaughlin, V.V. "*Endothelin receptor blockers in cardiovascular disease"* Circulation, Vol. (108), No.(18), pp. 2184-90. (2003).
23. Liu, J., Gong, H., Zhang, Z.T. and Wang Y. "*Effect of angiotensin II and angiotensin II type 1 receptor antagonist on the proliferation, contraction and collagen synthesis in rat hepatic stellate cells",* Chin Med J, Vol. (121) No. (2), pp.161-5. (2008).
24. Warner, F.J., Lubel, J.S., McCaughan, G.W. and Angus, P.W. "*Liver fibrosis: a balance of ACEs?*" Clin Sci, Vol. (113). No. (3), pp 109-18. (2007).
25. Abdulazeez, A.M., C.A. Awasum, Dogo, Y.S. and Abiayi, P.N. "*Effect of Peristrophe bicalyculata on Blood Pressure, Kidney and Liver Functions of Two Kidney One Clip (2K1C) Hypertensive Rats"* , British Journal of Pharmacology and Toxicology, Vol. (1), No. (2), pp.101-7. (2010).
26. Sookoian, S., Fernandez, M.A. and Castano, G. "*Effects of six months losartan administration on liver fibrosis in chronic hepatitis C patients: a pilot study",* World J Gastroenterol, Vol. (11), No.(48), pp. 7560-3. (2005).